Asian Journal of Plant Sciences

ISSN 1682-3974

science alert

ANSInet
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http://ansinet.com

Asian Journal of Plant Sciences 6 (1): 66-70, 2007
ISSN 1682-3974
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Actinomycetes Diversity in the Rhizosphere Soils of Different Medicinal Plants in Kolly Hills-Tamilnadu, India, for Secondary Metabolite Production

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Abstract: A study was undertaken to isolate actinomycetes from the soils which collected from Kolli Hills located at Salem District, Tamilnadu, India. The isolated actinomycetes were subjected to identify at the genus level and induce to produce bioactive secondary metabolites under *in vitro* condition. There were eight isolates obtained from different region of Kolli hills and studied for the detection of antimicrobial substances contained for it. These antimicrobial compounds were extracted and tested against some microorganisms like *Streptococcus faecalis*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Bacillus amyloliquefaciens*, *Staphylococcus aureus*, *Candida albicans* and *Cryptococcus neoformans*. The results showed that out of eight, five isolates were found to be of potential antagonists against pathogens and thus proving the production of secondary metabolites, which has the potential to control a variety of pathogenic organisms.

Key words: Actinomycetes, *Streptomyces*, antimicrobial activity

INTRODUCTION

Actinomycetes population has been identified as one of the major groups of the soil population. They are widespread in nature and found to be more in dry than wet soils (Wellington *et al*., 1994). Further, they can produce an array of secondary metabolites, many of which have antibacterial or antifungal properties. Infact, most antibiotics developed for human pharmaceutical uses are actinomycetes metabolites, many of them being derived from *Streptomyces* sp. (Goodfellow *et al*., 1987). *Streptomyces* are widely used in industries due to their ability to produce numerous chemical compounds including antibiotics, enzymes and anti-tumor agents (Berdy, 1995; Poornima and Ponmurugan, 2006).

The most promising role for secondary metabolites relies upon defense mechanisms. Inhibiting other, competing cells, would leave more nutrients for the survival of the secondary metabolites producing strain. Indeed many secondary metabolites show antibacterial or other inhibitory activities (anti-tumor, antifungal) or may function as herbicides (Sanglier *et al*., 1993; Ponmurugan and Poornima, 2006).

The present study aims at isolation and characterization of biologically diverse strains of actinomycetes from soil samples for the production of bioactive secondary metabolites. The antimicrobial activity of this genus was studied by performing test against a few microorganisms.

MATERIALS AND METHODS

The study was conducted at the Department of Biotechnology, Kaleeswari College, Sivakasi, Virudhunagar District, Tamilnadu, India, for a period of two years (2005-2006). Rhizosphere soil samples were collected from different medicinal plants grown in Kolli hills of Salem district, Tamilnadu, India. These samples were obtained at a depth of 6-10 cm in the rhizosphere regions of medicinally important plants such as Subabul (*Leucaena leucocephala*), Mahuva (*Madhuca induca*), Bhangria (*Eclypta alba*), Akven (*Gliricidia maculata*), Lawsone (*Lawsonia inermis*), Neem (*Azadirachta indica*), Karanj (*Pongamia globra*) and Paneer (*Chromolana oderata*). The soil samples were allowed to air dry at room temperature and various parameters like soil pH, total

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organic carbon (Walkey and Black, 1934), total nitrogen (AOAC, 1990) and available phosphorous (Jackson, 1973) were determined subsequently.

Isolation and enumeration of actinomycetes present in these soil samples was performed by serial dilution plate technique using Starch-casein nitrate agar medium. Biochemical characterization such as pigment production, starch hydrolysis, casein hydrolysis, catalase test, nitrate reduction, indole production, gelatin hydrolysis and hydrogen sulphide production were carried out to identify the name of actinomycetes.

In order to check the production of antibiotics by actinomycetes isolates, giant colony technique (Patel, 2003) and well-plat method (Rao *et al*., 2002) were used. The pure cultures of actinomycetes strains were inoculated into 100 mL of brain heart infusion broth taken in a 250 mL of conical flask. The flasks were kept in a shaker at 220 rpm for 15 days. The bioactive compounds were extracted from the medium after incubation period by filtration and centrifugation techniques. The extracts were tested against a few microorganisms using Muller Hinton agar medium (for bacteria) and yeast morphological agar medium (for yeast). The pathogenic microorganisms like *Streptococcus faecalis*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, *Candida albicans*, *Cryptococcus neoformans* and *Bacillus amyloliquefaciens* were selected for the present study. The inoculated plates were incubated at 37°C for 48 h and observed the zone of inhibition.

Amount of protein present in the cells of all the isolates of actinomycetes was estimated by Lowrey *et al*. (1951) method. 0.1 mL of culture filtrate, 5 mL of alkaline copper reagent and 0.5 mL of folin ciocalteau reagent (1:1) were mixed and incubated for 30 min under dark condition. Following incubation, the absorbance was read at 720 nm using a Spectronic 20D spectrophotometer against bovine serum albumin as a standard.

Thin layer chromatography method was employed to detect the presence of amino acids in the culture filtrate of *Streptomyces* isolates. A solvent system (mobile phase) contained a mixture of n-butanol, glacial acetic acid and water in the ratio of 4:1:1, Rf value was calculated based on the distance movement between the sample and solvent on the strip.

RESULTS AND DISCUSSION

Total number of actinomycete colonies was found to be more in the rhizosphere soils of Lawsone followed by Akven and lower in Mahuva plant species (Table 1). The population density of actinomycetes in the soil might be determined by the soil nutrients like total organic carbon, nitrogen and phosphorus (Table 2) reported by Baby *et al*. (2002). Most of the isolates tend to grow in fertile soils, which is an important characteristic feature of *Streptomyces* sp. (Stackebrandt *et al*., 1981) and with adequate source of carbon and nitrogen present in it that enhance the rate of degradation (Tien *et al*., 1987).

Table 1: Population density of actinomycetes in soil samples

Plant species	Population density (/g dry wt of soil)
Subabul	7.7×10^{-3}
Mahuva	4.3×10^{-4}
Bhangria	6.2×10^{-1}
Akven	10.9×10^{-1}
Lawsone	12.3×10^{-2}
Neem	8.3×10^{-3}
Karanj	7.5×10^{-3}
Paneer	6.5×10^{-3}

Table 2: Analysis of soil parameters and designation of actinomycetes isolates

pH	Total organic carbon (%)	Total nitrogen (%)	Available phosphorus	Designation of strains
6.7	0.0044	0.00011	0.0058	VA2
6.6	0.0057	0.00020	0.0009	CA1
6.5	0.0034	0.00008	0.0008	CA2
6.6	0.0036	0.00008	0.0007	MA3
7.7	0.0013	0.00022	0.0006	MA1
7.0	0.0027	0.00056	0.0066	MA4
7.2	0.0016	0.00056	0.0075	TA1
6.2	0.0043	0.00070	0.0068	TA2

There were eight isolates obtained from the rhizosphere soils of different medical plants grown in Kolli hills and were designated based on the filed/area (Table 1). This isolates were subjected to study their morphological physiological and biochemical characteristics (Table 3). The cultural characteristics of the actinomycetes isolates were studied by using different types of solid media. The results indicated that all the tested media were supported the growth significantly except glucose asparagines agar and glycerol asparagines agar medium (Table 4). Among the different types of carbon sources tested, L-arabinose, D-xylose and D-glucose were proved to be suitable for the growth of *Streptomyces* species (Table 5). Actinomycetes are nutritionally versatile being also to grow both a rich substrates and on these containing a minimum or even and apparent lack of nutrients (Wellington *et al*., 1994). The optimum temperature was found to be 25°C the pH was 6.5 for their maximal growth.

Based on the morphological, physiological and biochemical characteristics, the purified isolates of actinomycetes belonged to *Streptomyces* sp. As they showed good sporulation with compact, chalk-like dry colonies of different colony variation from pink to white colour. All the isolates were found to be gram-positive organisms and showed a branched mycelium in their cell morphology similar to fungal characters (Holt, 1989).

Table 3: Morphological, physiological and biochemical characterization of actinomycetes isolates

	Streptomyces strains used							
Parameters	VA2	CA1	CA2	MA3	MA1	MA4	TA1	TA2
Cell morphological	Spiral spore chain	Occur as rods	Cluster of spore chain	Spiral spore chain	Coiled spore chain	Occur as rods	Coiled spore chain	Cluster of spore chain
Colour of the mycelium/ Arial mycelium	Grayish white good	Pinkish white none	White none	Creamy yellow good	Radish white moderate	Pinkish white poor	Pink good	White moderate
Physiological characterization gram reaction	+	+	+	+	+	+	+	+
Pigment production	+	+	+	+	-	+	+	-
Starch hydrolysis	+	+	+	+	+	+	-	+
Casein hydrolysis	+	+	+	+	-	+	-	+
Catalase test	$+^1$	$+^1$	+	$+^1$	$+^1$	$+^1$	+	+
Nitrate reduction	+	+	+	+	-	+	+	-
Indole production	+	+	+	+	-	+	+	-
Gelatin hydrolysis	-	-	-	-	-	-	-	-
Hydrogen sulphite production	-	+	+	+	-	-	+	+

+ Positive; - Negative; $+^1$Weakly positive

Table 4: Cultural characteristics of actinomycete isolates

	Growth response							
Type of medium	VA2	CA1	CA2	MA3	MA1	MA4	TA1	TA2
Sucrose-nitrate agar		$+^1$	-	-	$+^1$	$+^1$	+	+
Glucose-asparagines agar		$+^1$	$+^1$	$+^1$	-	-	$+^1$	$+^1$
Glycerol-asparagines agar		$+^1$	+	$+^1$	-	-	$+^1$	$+^1$
Tyrosine agar		+	-	-	-	+	+	$+^1$
Nutrient agar		+	+	+	+	+	+	+
Yeast extract agar		+	+	+	$+^1$	+	+	+
Malt extract agar		+	+	$+^1$	+	+	-	$+^1$
Oatmeal agar		+	+	$+^1$	$+^1$	+	-	$+^1$

+ Positive; - Negative; $+^1$Weakly positive

Table 5: Physiological properties and utilization of carbon sources by actinomycetes isolates

	Actinomycetes isolates							
Parameters	VA2	CA1	CA2	MA3	MA1	MA4	TA1	TA2
Optimum temp. for growth (°C)	25	25	30	20	20	25	25	25
Optimum pH for growth	6.5	7.0	6.5	6.5	6.5	7.0	6.5	6.5
L-Arabinose	+	+	+	+	+	+	+	+
D-Xylose	+	+	+	+	+	+	+	+
D-Glucose	+	+	+	+	+	+	+	+
D-Fructose	+	-	-	+	+	+	-	-
Sucrose	+	-	-	-	-	-	-	-
I-Inositol	+	-	-	-	-	-	-	-
L-Rhamnose	+	+	-	-	-	-	+	+
Raffinose	+	-	-	+	+	+	-	-
D-Mannital	+	+	-	-	-	-	-	-
Cellulose	+	-	-	-	-	-	-	-

+ Positive; - Negative; $+^1$Weakly positive

The results on biochemical characterization indicated that pigment production was very well observed in most of the *Streptomyces* sp. Most of the isolates were efficient in hydrolyzing starch and casein (Ravel *et al*., 2000) except strain a few strains. Indole production was strictly negative but catalase was positive in all the isolates. Production of hydrogen sulphide, gelatin hydrolysis, casein and starch hydrolysis showed a positive result in majority of the isolates.

Protein concentration of the *Streptomyces* sp. showed that there was no significant difference observed among the isolates, however, it was more in MA4 followed by CA2 and MA3 (Table 6). As the amino acids are the precursors of metabolic activity, studies were undertaken to detect the presence of amino acids present in the culture filtrate of *Streptomyces* sp. Leucine, proline and tryptophan were the probable amino acids present in the culture filtrates (Table 6). Proteins are the structural

Table 6: Biochemical constituents of actinomycetes isolates

		Amino acids present in the culture filtrate	
Strains used	Protein concentration (%)	Rf value	Probable amino acids
VA2	0.344	0.090	Lysine
CA1	0.368	0.190	Glycine
CA2	0.440	0.460	Valine
MA3	0.442	0.410	Methionine
MA1	0.350	0.090	Lysine
MA4	0.548	0.625	Leucine
TA1	0.348	0.336	Proline
TA2	0.370	0.580	Phenylalanine

Table 7: Antimicrobial activity of *Streptomyces* strains against pathogenic microorganisms

	Actinomycetes isolates used							
Cultures used	VA2	CA1	CA2	MA3	MA1	MA4	TA1	TA2
Streptococcus faecalis MTTC 459	+	-	+	-	-	+	+	+
Pseudomonas aeruginosa MTCC 741	+	-	+	-	-	-	-	-
Bacillus amyloliquefaciens MTCC 610	+	-	+	+	+	-	+	-
Staphylococcus aureus MTCC 740	+	-	+	$+^1$	-	-	+	+
Escherichia coli MTCC 521	+	-	+	+	-	-	-	-
Candida albicans MTCC 3017	+	+	$+^1$	+	+	$+^1$	+	-
Cryptococcus neoformans MTCC 1215	+	$+^1$	+	+	-	-	-	-

+ Positive; - Negative; $+^1$Weakly positive

and functional unit of the cell thereby taking part in metabolic activities of the cell (Sanglier *et al*., 1993). Since the nature of the dibasic amino acids present in the cell wall of the organism that help to identify the actinomyceltes (Good fellow *et al*., 1987).

The results on the antimicrobial activity against standard pathogenic organisms showed that inhibition zone is formed around the pathogenic strain (Table 7). Similar result was observed by Zahner *et al*. (1979). The formation of inhibition zone around the pathogenic strain is due to the production of secondary metabolites by *Streptomyces* sp. (Demain, 1983; Sanglier *et al*., 1993). In the present study, out of eight isolates of *Streptomyces* strains used, 5 of the strains were found to be of Potential antagonists against pathogens.

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